Copper(II) Inhibition of the SARS-CoV-2 Main Protease

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Abstract.

In an analysis of the structural stability of the coronavirus main protease (Mpro), we identified regions of the protein that could be disabled by cobalt(III)-cation binding to histidines and cysteines [1]. Here we have extended our work to include copper(II) chelates, which we have docked to HIS 41 and CYS 145 in the Mpro active-site region. We have found stable docked structures where Cu(II) could readily bond to the CYS 145 thiolate, which would be lethal to the enzyme.

Introduction.

Many investigators are searching for a therapeutic agent to treat coronavirus infections. One target that has received much attention is the SARS-CoV-2 main protease (Mpro) [1-17], a homodimer whose structure has been determined (Figure 1) [7]. Of relevance here is that the search for inhibitors has focused almost entirely on organic molecules. Surprisingly, there has been little attention paid to inorganic complexes, a void that we are working hard to fill. It is our view that metal ion binding to histidines and cysteines could be lethal to the protease [1].

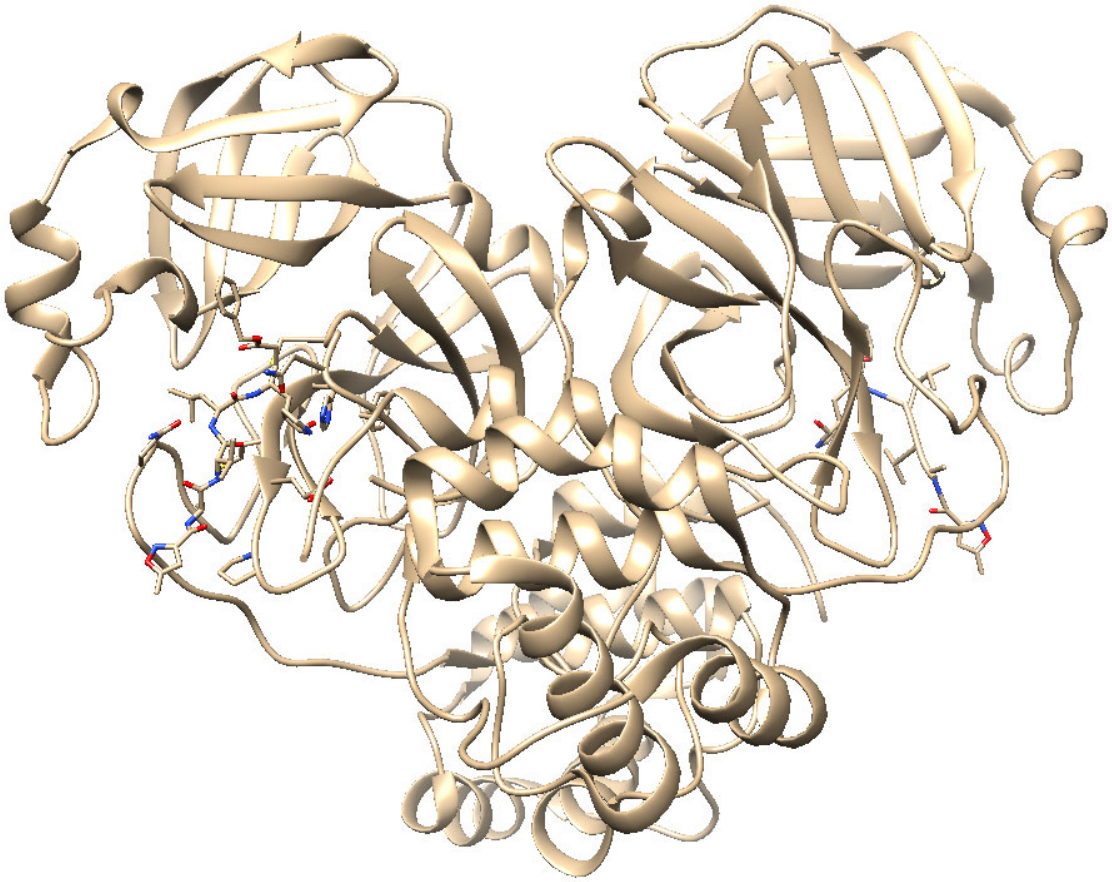

Figure 1. Chimera representation of the SARS-CoV-2 homodimer structure (PDB code: 6LU7).

Metal-ion Binding.

Of the seven histidines in the protease monomer, HIS 41 is in the most stable region of the native structure [1]. Metal-ion binding to the imidazole side chain of this histidine would break up the internal H-bond network (Figure 2), which would disable the enzyme. Other residues that could be targeted include HIS 163 and HIS 164, as they also are in relatively stable regions [1]. Among metal complex candidates that might bind in this region, $[Co(acacen)(NH_3)_2]^+$ is particularly attractive, as it is known to inhibit other proteases by HIS-imidazole displacement of one or both axial ammines [18,19].

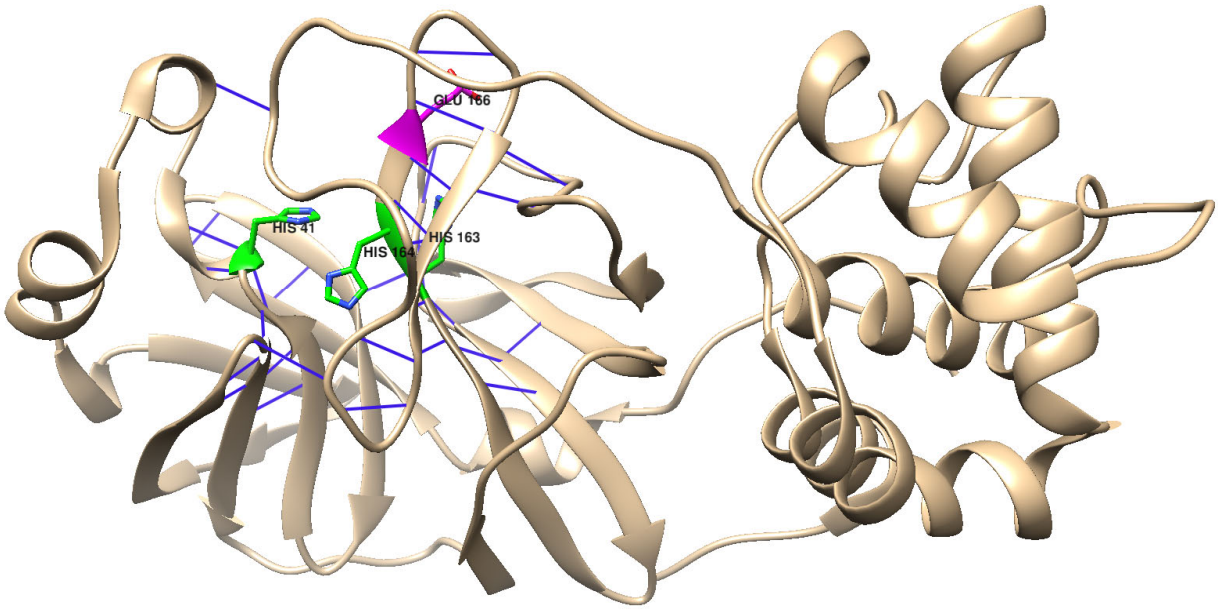

Figure 2. Chimera representation of the Mpro active-site H-bond network (blue lines); HIS 41, HIS 163, HIS 164, and GLU 166 are highlighted (PDB code 6Y2E).

We suggest that experiments using an excess of $[Co(acacen)(NH_3)_2]^+$ should be tried. If the protease is flooded with this cation, binding to at least 3 histidines could occur. Binding a cationic metal complex to several histidines would make the surface more hydrophilic, which in turn could trigger unfolding, as documented in our work on Co(III) binding to myoglobin [20].

In addition to experiments using an excess of $[Co(acacen)(NH_3)_2]^+$, other metal-ligand combinations could be effective. Particularly attractive are copper(II) complexes containing tridentate Schiff Base ligands (Figure 3), as they inhibit a thrombin protease [21].

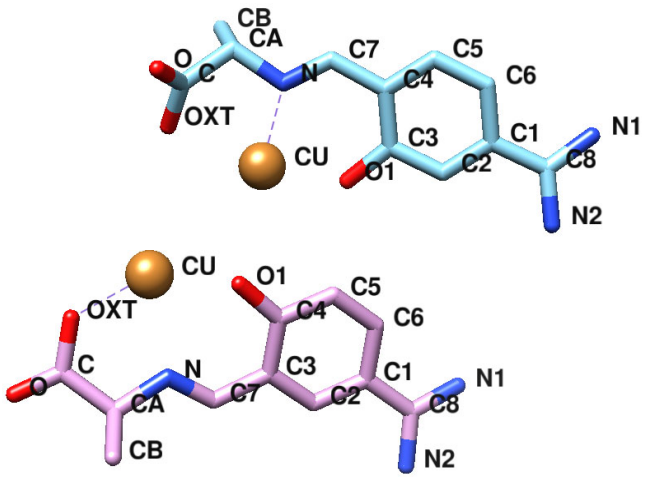

Figure 3. Structures of Schiff base copper(II) chelates:
top, [(para-amidinosalicylidene-l-alaninato)copper(II)], abbreviated Cu(II)Lp;
bottom, [(meta-amidinosalicylidene-l-alaninato)copper(II)], abbreviated Cu(II)Lm.
Chimera graphics of the ligands Cu(II)Lm and Cu(II)Lp obtained from PDB ID's:
1G3D and 1G3E respectively [21].

We successfully docked Cu(II)Lm near HIS 41 in the active-site region of the enzyme. Among the low-energy Cu(II)Lm/Mpro conjugates, one places the Cu atom near the CYS 145 thiolate sulfur at a distance (Cu-S) = 2.89 Å (Figure 4). [The Cu-S distance is 3.472 Å in the lowest energy docked structure (Figure S1).] In all docked structures near HIS 41, it is likely that protein motions would allow Cu(II) to bond directly to the CYS 145 thiolate, which in turn would be lethal to the protease. To explore steric effects on Cu(II)Lm/Mpro interactions, we docked less bulky analogues, Cu(II)Lm-methyl and Cu(II)Lm-mini, into the CYS 145 site (Figures 5, S2, and S3). The two oxygen donors are tightly bonded to Cu(II) in these docked sites, with Cu-S(CYS 145) = 3.11 Å in the structure shown in Figure 5. [Other Cu-S distances are 3.137 Å (Figure S2) and 3.098 Å (Figure S3).] Once again, small conformational motions might trigger Cu(II) ligation to the CYS 145 thiolate, which would disrupt protease function.

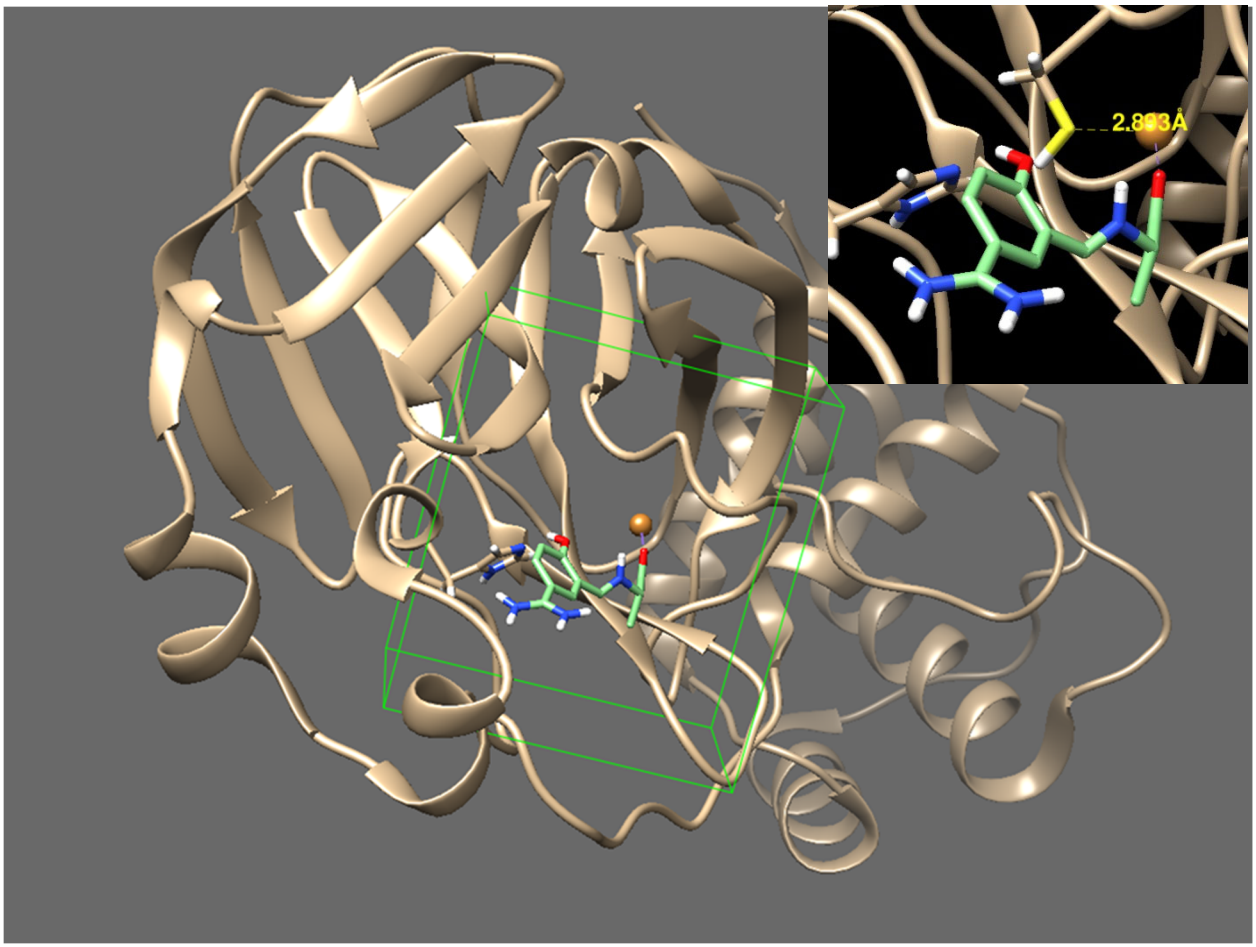

Figure 4. Chimera representation of Cu(II)Lm docked near HIS 41 in Mpro (PDB code 6Y2E). The inset shows the Cu-S(CYS 145) distance of 2.893 Å. The ligand and receptor files were prepared using the molecular docking program AutoDock Vina [22]. The amino acid HIS 41 is enclosed in the green box. AutoDock Vina predicts the bound conformations and the binding affinities (kcal/mol) within that space. The different poses were visualized via Chimera.

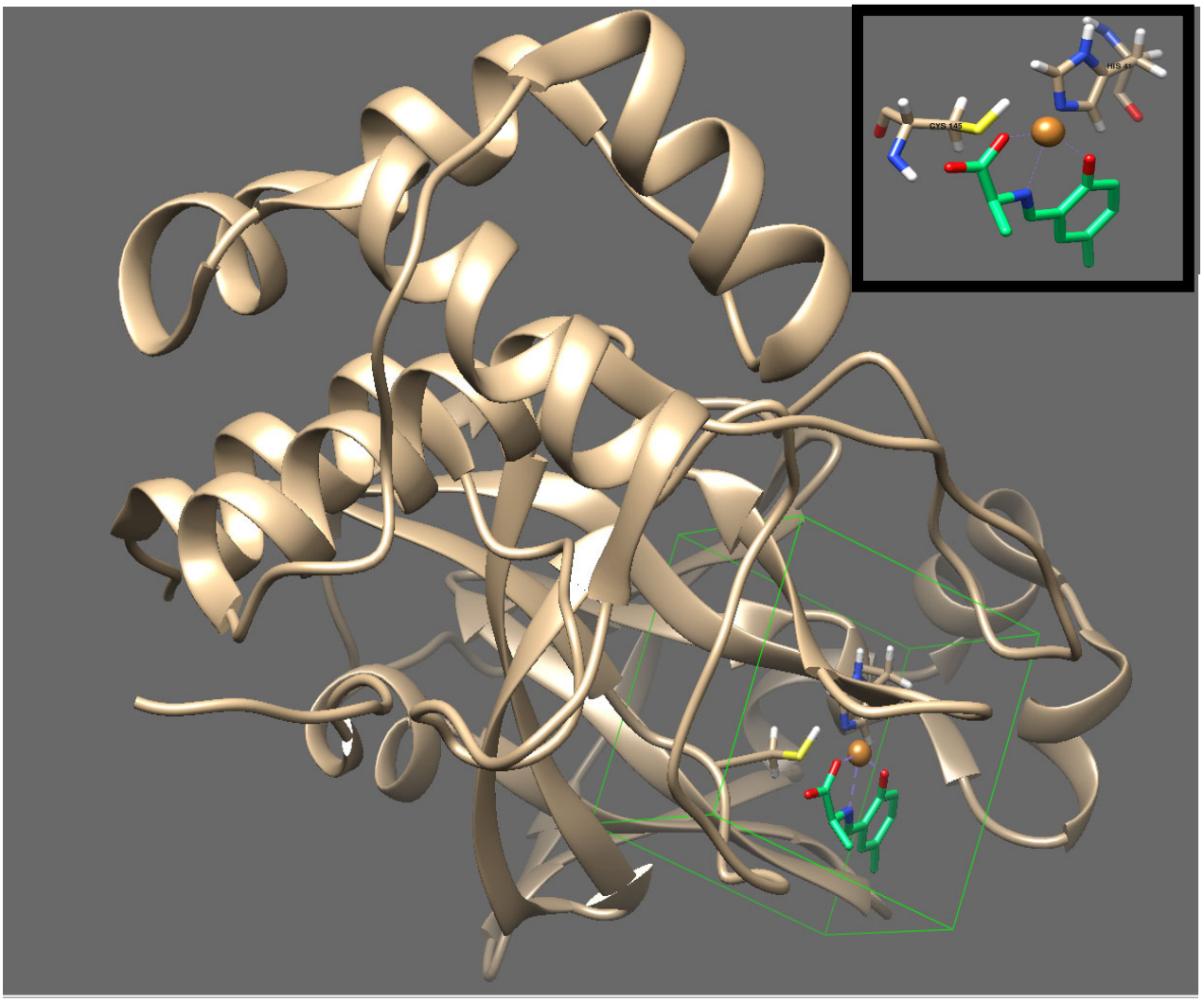

Figure 5. Chimera representation of Cu(II)Lm-methyl docked near CYS 145 in Mpro (PDB code 6Y2E); Cu(II) is 3.11 Å from the CYS 145 thiolate sulfur. The structure of Cu(II)Lm-methyl is shown in the inset. Docking details as in Figure 4.

Cobalt and copper ligation to cysteines other than CYS 145 also should be explored. Of the 12 cysteines in the protease monomer, arguably the most attractive target is CYS 44, which is on a very stable helix [1]. Displacement of an axial ammine in $[Co(acacen)(NH_3)_2]^+$ by the CYS 44 thiolate would trigger partial helical unfolding, which could disrupt protease function.

We also are studying the Spike protein (Figure 6), a trimer with many cysteines and histidines that are exposed to solvent [23]. We are testing the proposal that cobalt(III) or copper(II) binding to these residues would partially unfold the protein, including the region around GLY 614 in the D614G mutant.

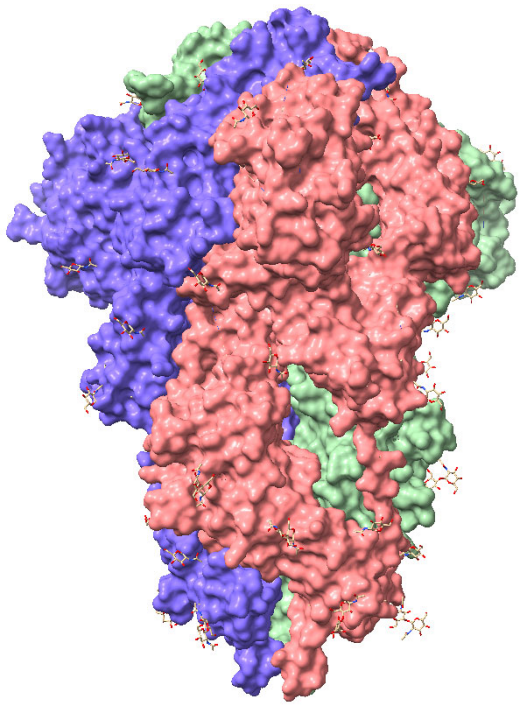

Figure 6. ChimeraX representation of SARS-CoV-2 spike glycoprotein (closed state), PDB ID: 6VXX. [23,24]

Concluding Remarks.

We have identified several very attractive histidine and cysteine targets for protease inhibiton by inorganic therapeutic agents. In one scenario, cobalt and copper binding to histidines could unfold the main protease. In others, the protease could be disabled by Cu-S bonding in Cu(II)Lm conjugates.

Acknowledgements.

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Supporting Information

Figure S1: Chimera representation of Cu(II)Lm docked near HIS 41 in Mpro (PDB code 6Y2E) with a binding energy of 6.8 kcal/mol. Inset: Cu(II) chelation; Cu-S(CYS 145) = 3.472 Å. The ligand and receptor files were prepared using the molecular docking program AutoDock Vina [22]. The amino acid HIS 41 is enclosed in the green box. AutoDock Vina predicts the bound conformations and the binding affinities (kcal/mol) within that space. The different poses were visualized via Chimera

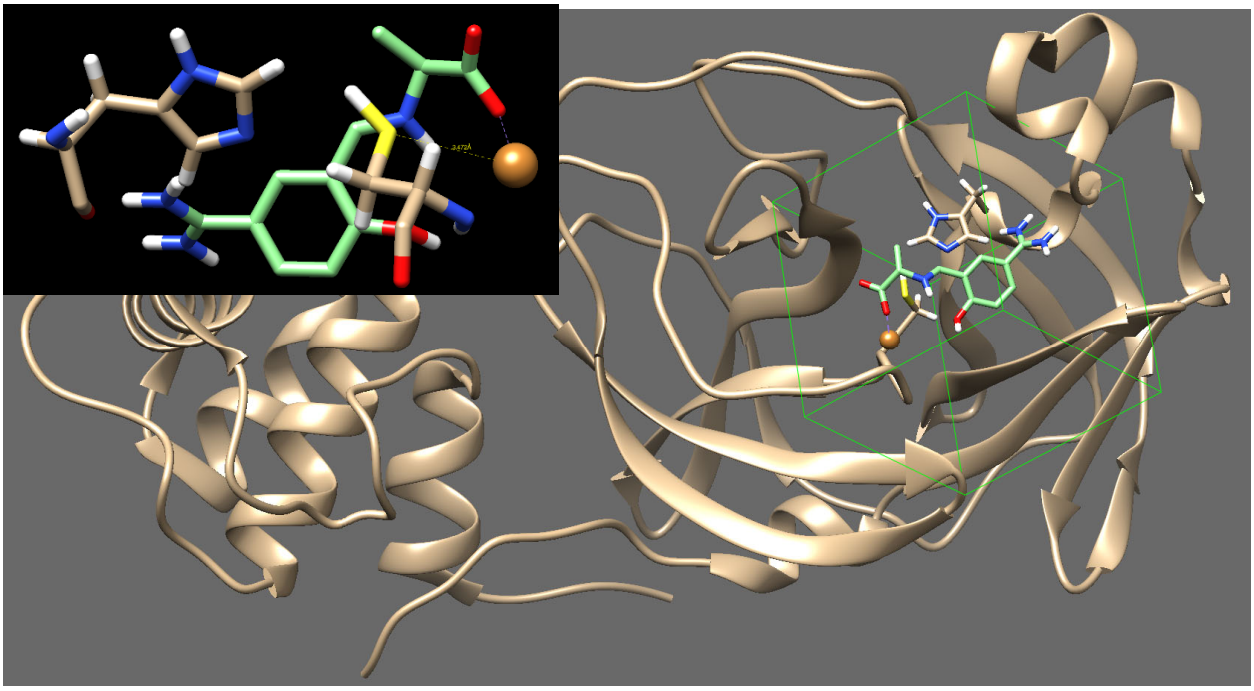

Figure S2. Chimera representation of Cu(II)Lm-methyl docked near HIS 41 in Mpro (PDB code 6Y2E) with a docking energy of 7.2 kcal/mol. Inset: Cu(II) chelation; Cu-S(CYS 145) = 3.137 Å. The ligand and receptor files were prepared as in Fig. S1.

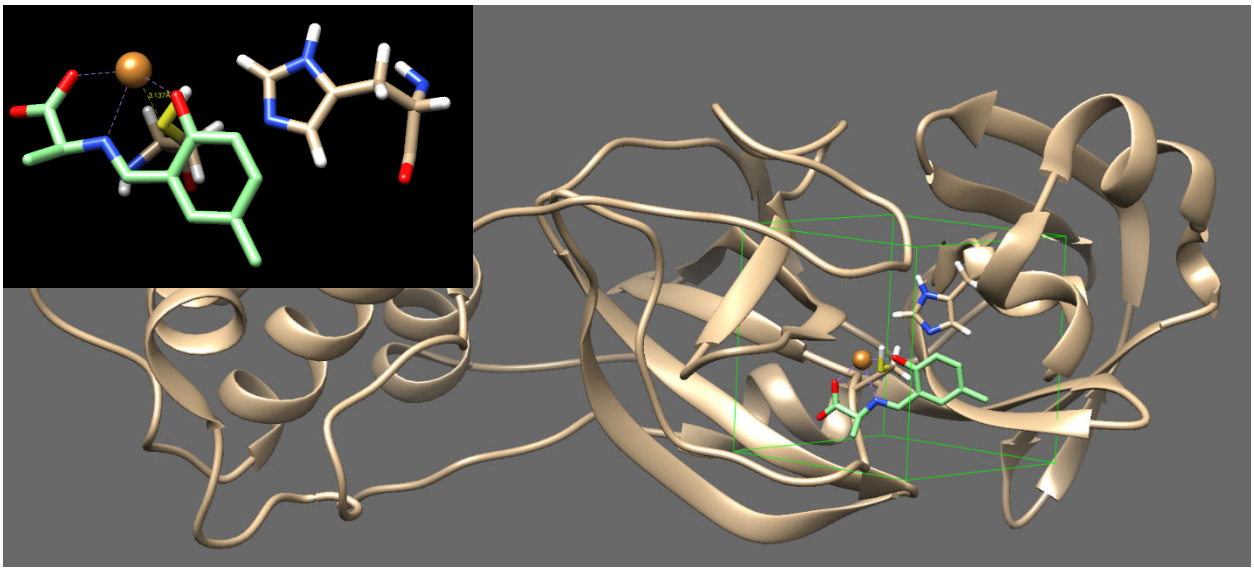

Figure S3: Chimera representation of Cu(II)Lm-mini docked near HIS 41 in Mpro (PDB code 6Y2E) with a binding energy of 6.2 kcal/mol. Inset: Cu(II) chelation; Cu-S(CYS 145) = 3.098 Å. The ligand and receptor files were prepared as in Fig. S1.